Current Biology 16, 1139–1146, June 6, 2006 DOI 10.1016/j.cub.2006.04.042

Report

Loss of Cell Polarity Drives Tumor Growth and Invasion through JNK Activation in *Drosophila*

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Summary

Apparent defects in cell polarity are often seen in human cancer [1, 2]. However, the underlying mechanisms of how cell polarity disruption contributes to tumor progression are unknown. Here, using a *Drosophila* genetic model for Ras-induced tumor progression, we show a molecular link between loss of cell polarity and tumor malignancy. Mutation of different apicobasal polarity genes activates c-Jun N-terminal kinase (JNK) signaling and downregulates the E-cadherin/β-catenin adhesion complex, both of which are necessary and sufficient to cause oncogenic Ras^{V12}-induced benign tumors in the developing eye to exhibit metastatic behavior. Furthermore, activated JNK and Ras signaling cooperate in promoting tumor growth cell autonomously, as JNK signaling switches its proapoptotic role to a progrowth effect in the presence of oncogenic Ras. Our finding that such context-dependent alterations promote both tumor growth and metastatic behavior suggests that metastasis-promoting mutations may be selected for based primarily on their growth-promoting capabilities. Similar oncogenic cooperation mediated through these evolutionarily conserved signaling pathways could contribute to human cancer progression.

Results

Most human cancers originate from epithelial tissues. These epithelial tumors, except for those derived from squamous epithelial cells, normally exhibit pronounced apicobasal polarity. However, these tumors commonly show defects in cell polarity as they progress toward malignancy [1, 2]. Although the integrity of cell polarity is essential for normal development [3], how cell polarity disruption contributes to the signaling mechanisms essential for tumor progression and metastasis is unknown. To address this, we used a recently established *Drosophila* model of Ras-induced tumor progression triggered by loss of cell polarity. This fly tumor model exhibits many aspects of metastatic behaviors observed in human malignant cancers, such as basement membrane degradation, loss of E-cadherin expression, migration, invasion, and metastatic spread to other organ sites [4]. In the developing eye tissues of these animals, loss of apicobasal polarity is induced by disruption of evolutionarily conserved cell polarity genes such as *scribble* (*scrib*), *lethal giant larvae* (*lgl*), or *discs large* (*dlg*), three polarity genes that function together in a common genetic pathway, as well as other cell polarity genes such as *bazooka*, *stardust*, or *cdc42* [3, 5]. Oncogenic Ras (Ras^{V12}), a common alteration in human cancers [4, 6], causes noninvasive benign overgrowths in these eye tissues [4]. Loss of any one of the cell polarity genes somehow strongly cooperates with the effect of Ras^{V12} to promote excess tumor growth and metastatic behavior [4, 7]. However, on their own, clones of *scrib* mutant cells are eliminated during development in a JNK-dependent manner; expression of Ras^{V12} in these mutant cells prevents this cell death [7].

To better quantify the metastatic behavior of tumors in different mutant animals, we focused our analysis on invasion of the ventral nerve cord (VNC), a process in which tumor cells leave the eye-antennal discs and optic lobes (the areas where they were born) and migrate to and invade a different organ, the VNC. We further confirmed that the genotypes associated with the invasion of the VNC in this study also resulted in the presence of secondary tumor foci at distant locations, although the number and size of these foci were highly variable, as previously reported [4].

Loss of Cell Polarity Activates JNK Signaling that Is Essential for Tumor Invasion

In analyzing the global expression profiles of noninvasive and invasive tumors induced in *Drosophila* developing eye discs, we observed that expression of the JNK phosphatase *puckered* (*puc*) was strongly upregulated in the invasive tumors (our unpublished data). Upregulation of *puc* represents activation of the JNK pathway in *Drosophila* [8–10]. We therefore utilized an enhancer-trap allele, *puc-LacZ* [11], to monitor the activation of JNK signaling in invasive tumor cells. Strong ectopic JNK activation was present in invasive tumors (Figures 1D–1F), while only a slight expression of *puc* was seen in restricted regions of Ras^{V12}-induced noninvasive overgrowth (Figures 1A–1C). Intriguingly, more intense JNK activation was seen in tumor cells located in the marginal region of the eye-antennal disc and tumor cells invading the VNC (Figures 1D–1I, arrowheads, and also see Figure S1 in the Supplemental Data available with this article online). Analysis of clones of cells with a cell polarity mutation alone revealed that JNK signaling was activated by mutation of cell polarity genes (Figures 1J–1O). Notably, JNK signaling was not activated in a strictly cell-autonomous fashion (Figures 1M–1O, arrowheads). JNK activation in these cells was further confirmed by anti-phospho-JNK antibody staining that detects activated JNK (see Figure S2).

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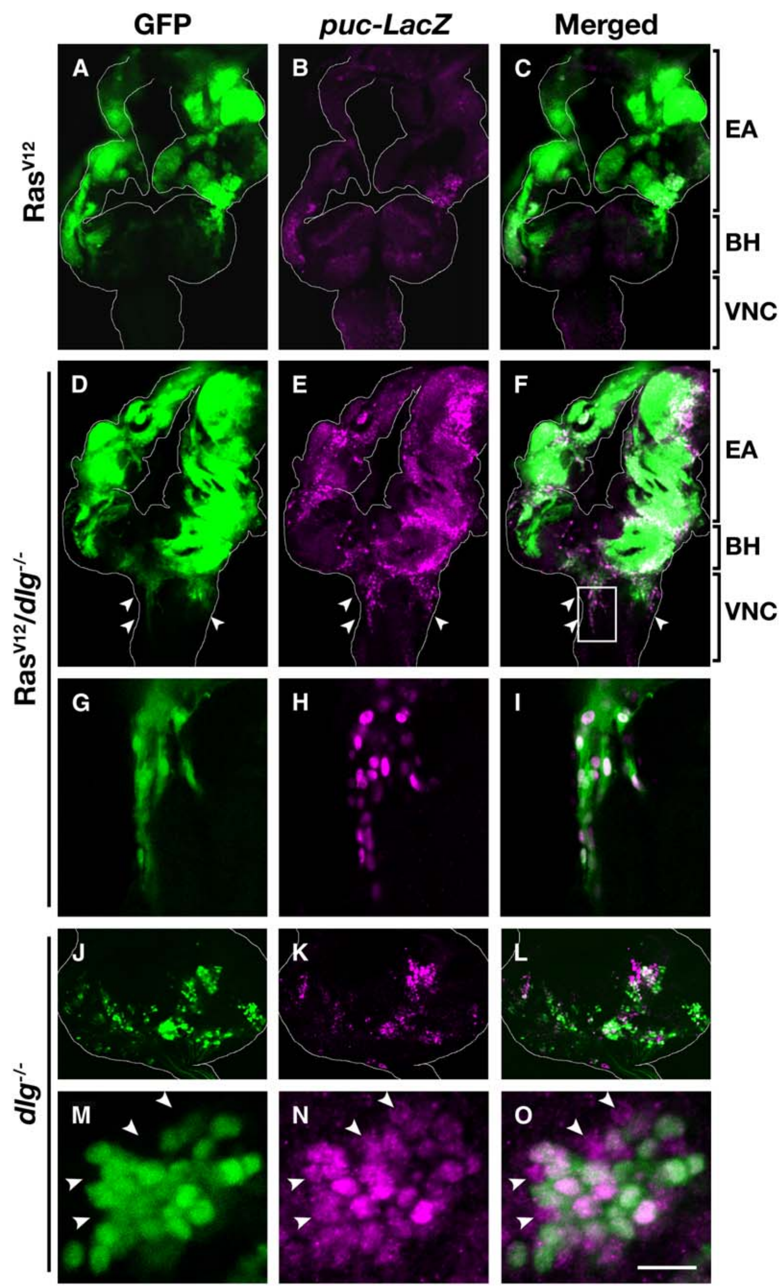

Figure 1. Invading Cells Are Activating JNK, which Is Triggered by Loss of Cell Polarity

JNK activation was determined by anti-β-galactosidase staining (magenta) in the genetic background of *puc-LacZ* in GFP-labeled Ras^{V12} benign overgrowth (A–C), Ras^{V12}/$dlg^{-/-}$ invasive tumors (D–I), or cell polarity-deficient $dlg^{-/-}$ clones (J–O) (green). Confocal images of the day 6 cephalic complexes, which include the eye-antennal discs (EA), brain hemispheres (BH), the VNC (see also Figure 2) (A–I), and the day 4 eye discs (J–O) are shown. JNK is strongly activated in invading cells in the BH or the VNC ([D]–[I] and arrowheads. (G)–(I) are high-magnification images shown by a square in (F). High magnification of $dlg^{-/-}$ clones shows some non-cell-autonomous upregulation of *puc* ([M]–[O], arrowheads; scale bar, 10 μm). n > 10 for each genotype. See Supplemental Data for genotypes.

To examine the contribution of JNK activation to metastatic behavior, we blocked the JNK pathway by overexpressing a dominant-negative form of *Drosophila* JNK (Bsk^{DN}). As previously reported [4], clones of cells mutant for *scrib*, *lgl*, or *dlg* do not proliferate as well as wild-type clones (Figures 2A–2D), while combination of these mutations with Ras^{V12} expression resulted in massive and metastatic tumors (Figures 2G, 2G′, 2I, 2I′, 2K, and 2K′). Strikingly, inhibition of JNK activation by Bsk^{DN} completely blocked the invasion of the VNC (Figures 2G′–2L′), as well as secondary tumor foci formation (see Figure S3). *Drosophila* has two homologs of TRAF proteins (DTRAF1 and DTRAF2), which mediate signals from cell surface receptors to the JNK kinase cascade in mammalian systems [12]. We found that RNAi-mediated inactivation of DTRAF2, but not DTRAF1, in the tumors strongly suppressed their metastatic behavior (Figures 2M′ and 2N′). Inactivation of dTAK1, a *Drosophila* JNK kinase kinase (JNKKK), or Hep, a JNKK, also suppressed metastatic behavior (Figures 2O′ and 2P′). *Drosophila* has two known cell surface receptors that act as triggers for the JNK pathway, Wengen (TNF receptor) and PVR (PDGF/VEGF receptor) [13, 14]. Intriguingly, we found that RNAi-mediated inactivation of Wengen partially suppressed tumor invasion (Figure 2Q′). Inactivation of PVR, on the other hand, did not show any suppressive effect on metastatic behavior (Figure 2R′). We also found that the metastatic behavior of Ras^{V12}-expressing tumors that were also mutated for one of three other cell polarity genes, *bazooka*, *stardust*, or *cdc42*, was also blocked by Bsk^{DN} (data not shown). These data indicate that loss of cell polarity contributes

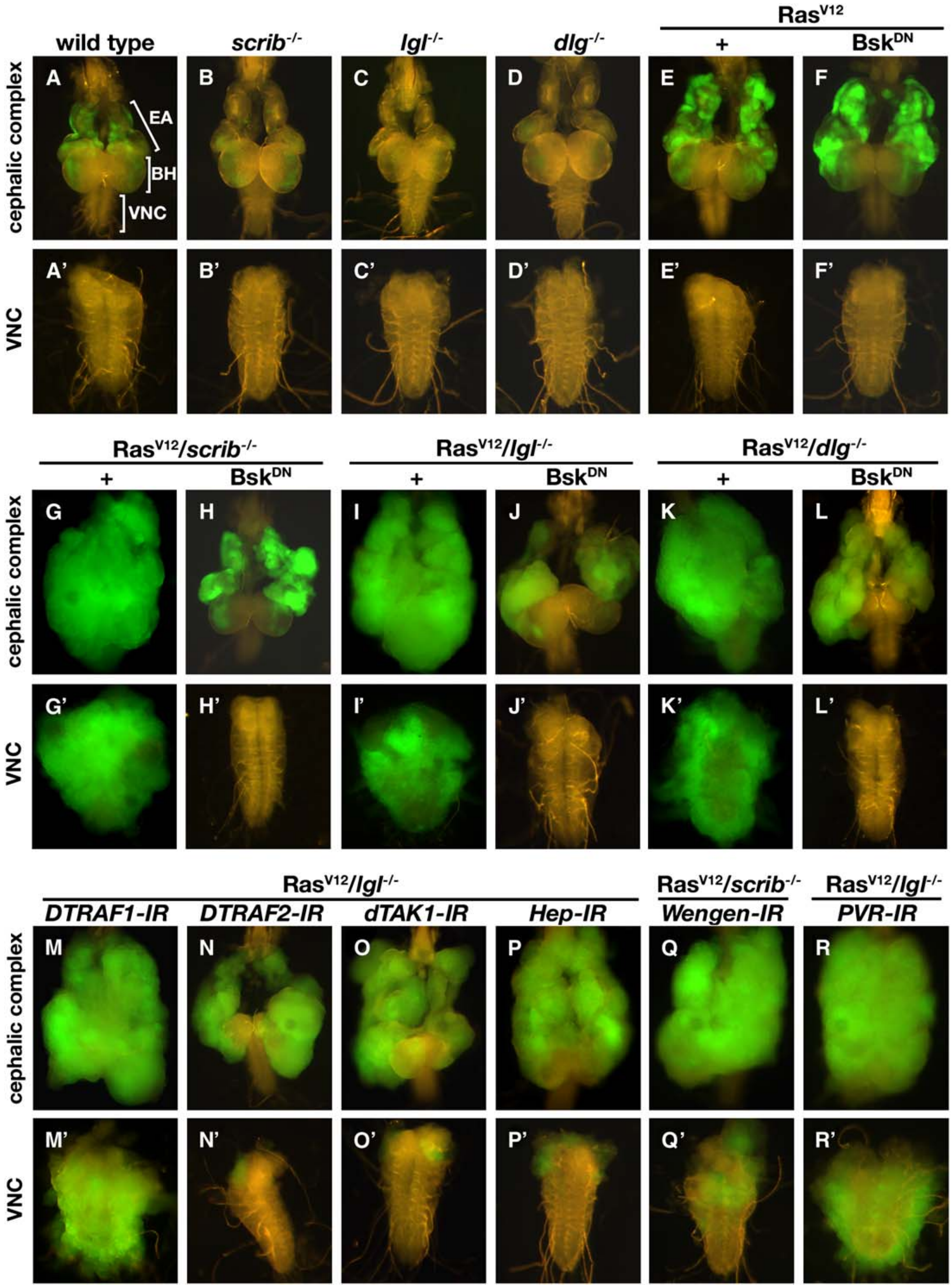

Figure 2. JNK Pathway Activation Is Essential for Both Accelerated Tumor Growth and Metastatic Behavior

Dorsal views of the cephalic complexes (A–R) and VNCs (A′–R′) are shown. Wild-type clones do not show any growth advantage (A). Clones with cell polarity mutation alone result in smaller clones (B–D). Ras^{V12}-expressing clones show a moderate growth advantage (E) but never invade the VNC (E′). Massive tumor growth and invasion to the VNC caused by Ras^{V12}/*scrib*$^{-/-}$ (G and G′), Ras^{V12}/*lgl*$^{-/-}$ (I and I′), or Ras^{V12}/*dlg*$^{-/-}$ (K and K′) are strongly suppressed by coexpression of Bsk^{DN} (H, H′, J, J′, L, and L′), RNAi construct of DTRAF2 (N and N′), dTAK1 (O and O′), or Hep (P and P′), partially suppressed by RNAi of Wengen (Q’, invasion only), but not by RNAi of DTRAF1 or PVR (M, M′, R, and R′). Bsk^{DN} does not suppress Ras^{V12}-stimulated growth (F). All genotypes displayed 100% phenotypic penetrance (n > 10 for each genotype) except for three genotypes with RNAi constructs, DTRAF2-IR (N and N′), dTAK1-IR (O and O′), and Wengen-IR (Q′), which still showed 89% (n = 19), 63% (n = 41), and 48% (n = 31) penetrance, respectively. See Supplemental Data for genotypes.

to metastatic behavior by activating the evolutionarily conserved JNK pathway.

Activation of JNK and Inactivation of E-Cadherin/ β-Catenin Are Downstream Events of Cell Polarity Disruption that Trigger Tumor Invasion

We next examined whether JNK activation is sufficient to trigger metastatic behavior in Ras^{V12}-induced benign tumors. Two genetic alterations can be used to activate JNK in *Drosophila*. First, JNK signaling can be activated by overexpression of Eiger, a *Drosophila* TNF ligand [15, 16]. While mammalian TNF superfamily proteins activate both the JNK and NFκB pathways, Eiger has been shown to specifically activate the JNK pathway through dTAK1 and Hep [15]. Indeed, the eye phenotype caused by Eiger overexpression could be reversed by blocking JNK through Bsk-IR (Bsk-RNAi) (Figures 3N and 3O). Second, overexpression of a constitutively activated form of Hep (Hep^{CA}) can also activate JNK signaling [8]. However, the eye phenotype caused by Hep^{CA} overexpression was only slightly suppressed by Bsk-IR (Figures 3P and 3Q), suggesting that Hep^{CA} overexpression may have additional effects other than JNK activation. We therefore used Eiger overexpression to activate JNK in Ras^{V12}-induced benign tumors and found that the Ras^{V12}+Eiger-expressing tumor cells did not result in the invasion of the VNC (Figure 4P and data not shown). This indicates that loss of cell polarity must induce an additional downstream effect(s) essential for metastatic behavior. A strong candidate for the missing event is downregulation of the E-cadherin/catenin adhesion complex, since this complex is frequently downregulated in malignant human cancer cells [17, 18] and is also downregulated by loss of cell polarity genes in *Drosophila* invasive tumors [4] (and data not shown). In addition, it has been recently reported that higher motility of mammalian *scrib* knockdown cells can be partially rescued by overexpression of E-cadherin-catenin fusion protein [19], suggesting a role of E-cadherin in preventing polarity-dependent invasion. Furthermore, overexpression of E-cadherin blocks metastatic behavior of Ras^{V12}/*scrib*$^{-/-}$ tumors [4], indicating that loss of E-cadherin is essential for inducing tumor invasion in this model. We found that loss of the *Drosophila E-cadherin* homolog *shotgun* (*shg*), combined with the expression of both Ras^{V12} and Eiger, induced the invasion of the VNC (88% penetrance, n = 119) (Figure 3D′, arrowheads). Intriguingly, loss of *shg* in Ras^{V12}-expressing clones also showed a weak invasive phenotype at lower penetrance (20%, n = 101) (Figure 3B′, arrowheads). In agreement with the essential role of JNK in tumor invasion, clones of *shg*$^{-/-}$ cells weakly upregulated *puc* expression (Figures 3G–3I). We further found that JNK activation in *dlg*$^{-/-}$ clones was not blocked by overexpression of E-cadherin (Figures 3J–3L), suggesting that mechanism(s) other than loss of E-cadherin also exist for inducing JNK activation downstream of cell polarity disruption. The metastatic behavior of Ras^{V12}+Eiger/*shg*$^{-/-}$ tumors was completely blocked by coexpression of Bsk^{DN} (Figure 3E′), indicating a cell-autonomous requirement of JNK activation for this process. Furthermore, we found that loss of the *β-catenin* homolog *armadillo* also induced metastatic behavior in Ras^{V12}-induced benign tumors (see Figure S4).

On the other hand, overexpression of Hep^{CA} in Ras^{V12}/*shg*$^{-/-}$ cells resulted in neither enhanced tumor growth nor metastatic behavior (Figures 3F and 3F′), which is consistent with our data (Figures 3N–3Q) and the previous report [20]. Together, these results suggest that, although the Ras^{V12}+Eiger/*shg*$^{-/-}$ does not completely phenocopy the effect of Ras^{V12}/*scrib*$^{-/-}$, activation of JNK signaling and inactivation of the E-cadherin/catenin complex are the downstream components of cell polarity disruption that trigger metastatic behavior in Ras^{V12}-induced benign tumors.

JNK Activation Is Essential for Accelerated Tumor Growth

Aside from its evolutionarily conserved role in cell migration and invasion [10, 21], JNK signaling is also a potent activator of cell death in *Drosophila* and mammals [8, 15, 22]. Although Ras^{V12}-expressing tissues showed a weak and restricted activation of JNK at later stages of development, mutation of cell polarity genes in combination with Ras^{V12} expression constitutively activated JNK signaling (see Figure S5). Striking acceleration of tumor growth occurred during days 5 and 6, and these tumors outcompeted surrounding wild-type tissues, resulting in a loss of the unlabeled wild-type cells and a dramatic increase in the GFP-expressing mutant tissue (see Figure S5). The activated JNK was correlated with this accelerated tumor growth, suggesting that JNK signaling may play a role in tumor growth (see Figure S5). Indeed, in addition to blocking metastatic behavior, inactivation of JNK pathway components strongly suppressed the accelerated tumor growth caused by cell polarity disruption (Figures 2G–2P). These results reveal that JNK signaling activated by loss of cell polarity also stimulates tumor growth.

Tumor Growth and Invasion Are Separable Processes

Since JNK signaling was required for both tumor growth and invasion, we next asked whether these two phenotypes were separable processes. To address this, we analyzed different types of tumors caused by alterations in genes involved in cell proliferation, growth, and cell polarity. Day 6 Ras^{V12}/*scrib*$^{-/-}$ tumors showed moderate tumor growth and VNC invasion phenotypes (Figures 4A and 4A′). Loss of the *Akt* gene, a component of insulin growth signaling, considerably reduced the tumor load of Ras^{V12}/*scrib*$^{-/-}$ animals but did not impair metastatic behavior (Figures 4B and 4B′). On the other hand, overexpression of Akt, combined with mutations in both the *scrib* gene and the *lats* gene, a potent tumor suppressor [23], did not cause metastatic behavior despite accelerated tumor growth comparable to Ras^{V12}/*scrib*$^{-/-}$ (Figures 4C and 4C′). In addition, although Ras^{V12}/*Tsc1*$^{-/-}$ mutant cells resulted in extremely large tumors, these tumor cells never exhibited metastatic behavior (Figure 4D). These data indicate that tumor growth and invasion are separable processes in this model system.

JNK Switches Its Proapoptotic Role to a Progrowth Effect in the Presence of Oncogenic Ras

Consistent with previous reports [7, 20], we found that JNK signaling was indeed activated in polarity-deficient

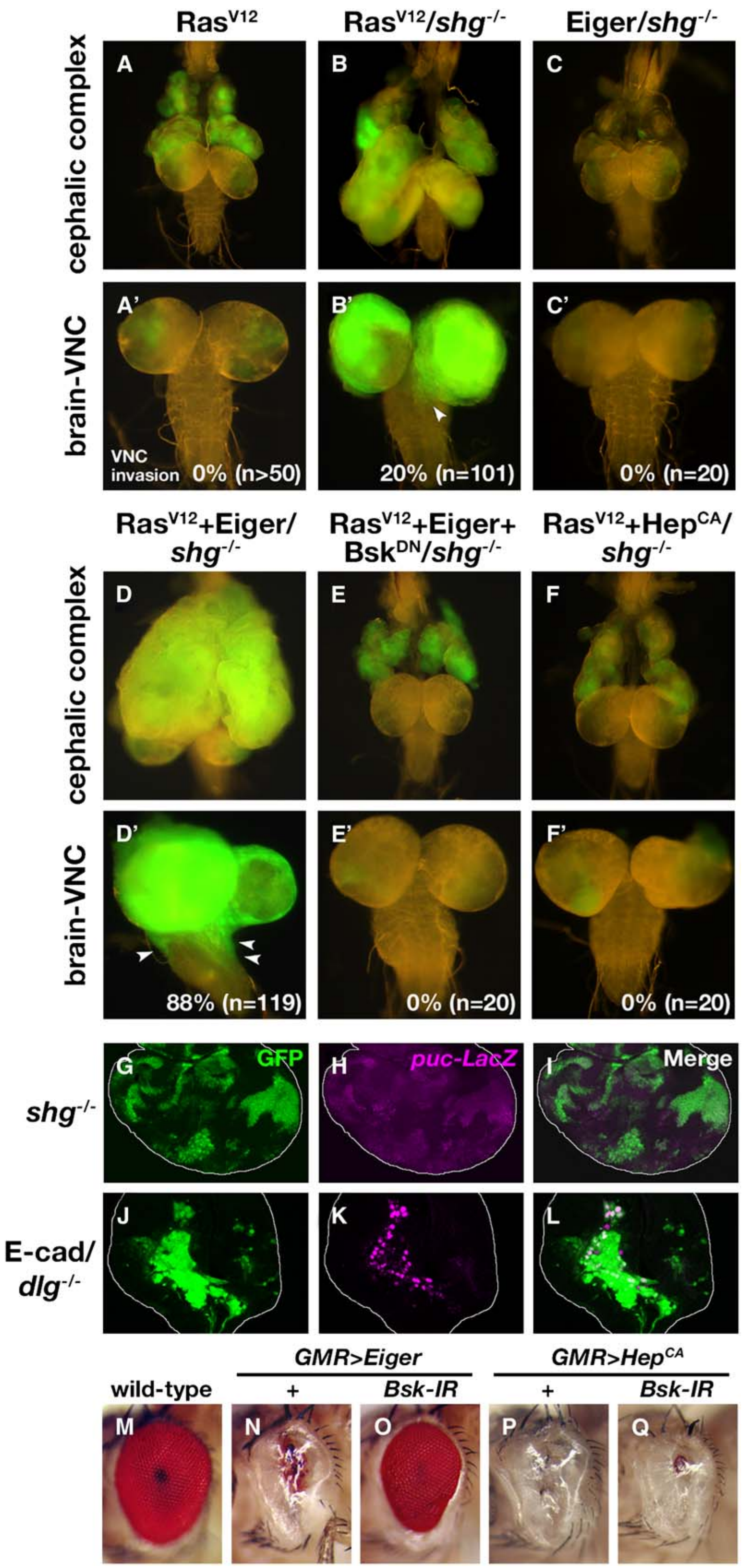

Figure 3. Activation of JNK Signaling and Inactivation of E-Cadherin Are Downstream Elements of Cell Polarity Disruption that Trigger Metastatic Behavior and Accelerated Growth in Ras^{V12}-Induced Tumors

(A–F and A′–F′) Cephalic complexes (A–F) and brain-VNCs (A′–F′) with GFP-labeled (green) clones of Ras^{V12} (A and A′), Ras^{V12}/*shg*$^{-/-}$ (day 12) (B and B′), Eiger/*shg*$^{-/-}$ (C and C′), Ras^{V12}+Eiger/*shg*$^{-/-}$ (day 12) (D and D′), Ras^{V12}+Eiger+Bsk^{DN}/*shg*$^{-/-}$ (E and E′), and Ras^{V12}+Hep^{CA}/*shg*$^{-/-}$ (F and F′) are shown. The penetrance of VNC invasion phenotype for each genotype is also shown.

(G–L) Confocal images of anti-β-galactosidase staining (magenta) of the eye discs with clones (green) of *shg*$^{-/-}$ (G–I) or E-cadherin/*dlg*$^{-/-}$ (J–L) in the genetic background of *puc-LacZ* is shown.

(M–Q) Wild-type eye (M) and eyes overexpressing Eiger (N and O) or Hep^{CA} (P and Q) without (N or P) or with (O or Q) RNAi construct of Bsk (Bsk-IR) are shown. All flies were raised at 25ºC, except that GMR>Hep^{CA} flies (P) were raised at 18ºC because of their pupal lethality at 25ºC. See Supplemental Data for genotypes.

cells (Figures 1J–1O), and acridine orange staining revealed that most of these cells were dying (Figures 4E–4G). Interestingly, ectopic cell death was mostly blocked within clones of polarity-deficient cells also expressing Ras^{V12} (Figures 4H–4J) [7], despite strong JNK activation (Figures 4K–4M). In addition, coexpression of Ras^{V12} and Eiger, a potent inducer of cell death [15, 16] (Figure 3N), resulted in accelerated tumor growth (Figure 4P),

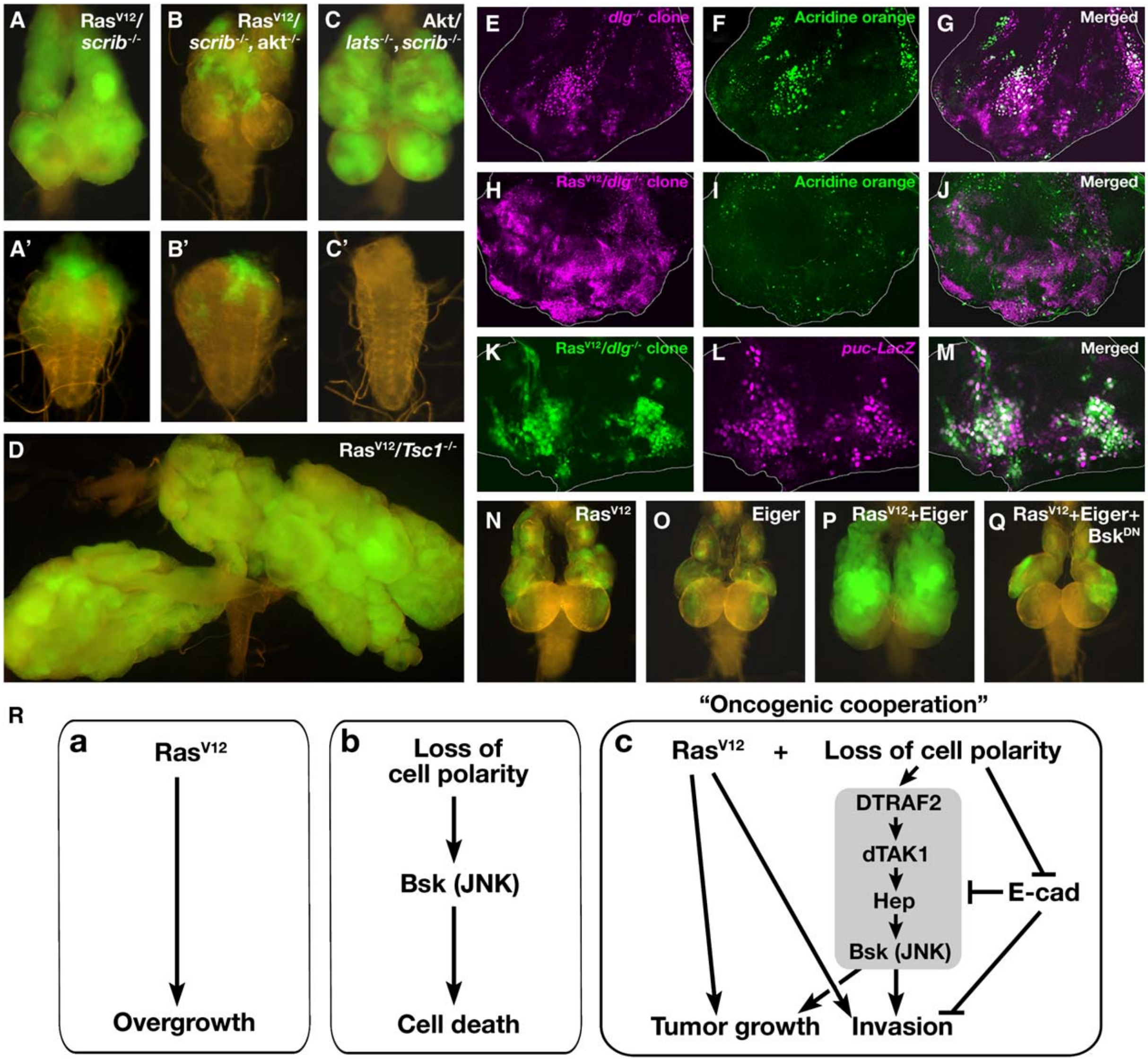

Figure 4. JNK Signaling Switches Its Role from Proapoptotic to Progrowth in the Presence of Oncogenic Ras

(A–D) Tumor growth and invasion are separable processes. Dorsal views of the cephalic complexes (A–D) and VNCs (A′-C′) with different types of tumors are shown. Invasion of the VNC can be seen without significant tissue overgrowth (B and B′), while accelerated growth is not sufficient to cause VNC invasion (C, C′, and D′). n > 10 for each genotype.

(E–Q) JNK signaling switches its proapoptotic role to a progrowth effect in the context of Ras activation. Dying cells were visualized by acridine orange staining (green) in day 4 eye discs with *dlg*$^{-/-}$ (E–G) or Ras^{V12}/*dlg*$^{-/-}$ (H–J) clones (magenta). Red fluorescent protein (RFP) was used for labeling clones to distinguish them from the green staining of acridine orange. JNK activation was detected by anti-β-galactosidase staining (magenta) within Ras^{V12}/*dlg*$^{-/-}$ clones (green) in day 4 eye discs (K–M). More than 50 clones are analyzed in each experiment in E-M. (N–Q) Cephalic complexes with GFP-labeled clones of Ras^{V12} (N), Eiger (O), Ras^{V12}+Eiger (P), and Ras^{V12}+Eiger+Bsk^{DN} (Q) are shown. At days 10–14, combined expression of Ras^{V12} and Eiger using a weak or a strong allele of UAS-Eiger resulted in massive tumor growth (P) with 24.1% (n = 245) or 44.1% (n = 229) penetrance, respectively. n > 10 for each genotype. See Supplemental Data for genotypes.

(R) A model for polarity disruption-induced oncogenic cooperation among the Ras, JNK, and E-cadherin pathways that causes tumor growth and invasion. Clones of cells expressing Ras^{V12} alone cause noninvasive benign overgrowth (Ra). Clones of cells with disrupted cell polarity alone result in JNK activation and subsequent cell death (Rb). Loss of cell polarity in the presence of oncogenic Ras results in accelerated tumor growth and invasion through cooperation of Ras and JNK activation and E-cadherin (E-cad) inactivation (Rc). The proapoptotic effect of JNK signaling (Rb) is converted to a progrowth effect in the presence of oncogenic Ras (Rc).

although neither Ras^{V12} alone nor Eiger alone caused dramatic overgrowth (Figures 4N and 4O). This massive overgrowth was completely blocked by coexpression of Bsk^{DN} (Figure 4Q). Moreover, stimulation of JNK signaling by expressing Eiger dramatically enhanced tumor growth of Ras^{V12}/*shg*$^{-/-}$ tissues (compare Figures 3B and 3D), although Eiger/*shg*$^{-/-}$ clones were very small (Figures 3C), probably because of cell death of these mutant clones. The accelerated growth of the Ras^{V12}+Eiger/*shg*$^{-/-}$ tumors was again completely blocked by Bsk^{DN} (Figure 3E). Together, these data indicate that, in the context of oncogenic Ras, JNK activation is the primary mediator of tumor growth downstream of cell polarity disruption. Our observations suggest that JNK signaling switches its proapoptotic role to a progrowth effect in the presence of oncogenic Ras, and that the dramatic tumor growth is caused by cooperation between oncogenic Ras and JNK signaling.

Discussion

Our study provides a molecular link between loss of cell polarity and tumor malignancy, namely activation of JNK signaling and inactivation of the E-cadherin/catenin complex in the context of oncogenic Ras activation. Although Ras^{V12} alone only induces noninvasive overgrowth, and loss of cell polarity alone results in JNK-mediated cell death, the combination of these two alterations promotes both tumor growth and invasion through oncogenic cooperation (Figure 4R). Thus, the tumor-promoting alterations caused by loss of cell polarity do not function alone and rather act as oncogenic Ras modifiers or "oncomodifiers."

The JNK signaling is essential for a variety of biological processes such as morphogenesis, cell proliferation, migration, invasion, and cell death [22]. Genetic studies in *Drosophila* have demonstrated that JNK signaling is essential for epithelial cell movements and invasive behavior during normal development [10, 21, 22]. A genetic study in mice revealed that TNF-triggered JNK signaling stimulates epidermal proliferation [24]. These studies suggest that JNK may play an important role in tumorigenesis, tumor growth, and metastasis. Indeed, a substantial body of evidence indicates that JNK activation and c-Jun phosphorylation play important roles in cancer development (e.g., reviewed in [25]). In mammalian cell culture systems, Ras acts cooperatively with JNK or c-Jun to enhance cellular transformation [26–28]. Furthermore, knockin mice expressing a mutant form of c-Jun ($Jun^{S63A,S73A}$) suppress development of skin tumors in response to Ras activation [29] and also block development of intestinal epithelial cancers caused by *APC* mutation [30]. Moreover, liver-specific inactivation of c-Jun impairs development of chemically induced hepatocellular carcinomas [31]. Furthermore, JNK signaling is activated in many tumor types (e.g., reviewed in [32]). On the contrary, however, it has been also shown that JNK functions as a negative regulator for tumor development in Ras/p53-transformed fibroblasts [33]. Thus, the role of JNK signaling seems to be highly dependent on cellular context, and, to our knowledge, our study provides the first evidence for a cell-autonomous oncogenic cooperation between JNK and Ras signaling that promotes tumor growth and malignancy.

How is JNK signaling activated? Loss of cell polarity may directly influence activity of a JNK pathway component. Alternatively, cell polarity defects may activate a cell surface receptor that triggers JNK signaling. Our genetic analysis of multiple JNK pathway components suggests that the pathway is activated through a cell surface receptor, Wengen. It would be interesting to further investigate whether mislocalization or disregulation of Wengen, which should be normally tightly regulated in polarized epithelial cells, results in stimulation of JNK pathway signaling.

Our discovery that metastasis-promoting alterations (i.e., JNK activation) also increase tumor growth may explain why tumor cells acquire such mutations; that is, they primarily provide a selective advantage in tumor growth. Given that cell polarity defects are frequently associated with human tumor malignancy, and that the pathways identified in *Drosophila* are evolutionarily conserved, similar molecular mechanisms could be involved in human tumor progression. It would be particularly interesting to study these processes in human tumors with high frequencies of Ras mutations. If such processes prove conserved, components of these pathways, especially JNK signaling, could serve as potential therapeutic targets against such cancers.

Experimental Procedures

Fly Strains and Generation of Clones

Fluorescently-labeled noninvasive or invasive tumors were produced in the eye discs as previously described [4] using the following strains: Tub-Gal80, FRT19A; eyFLP5, Act>y^+>Gal4, UAS-GFP (19A tester), y,w, eyFLP1; Tub-Gal80, FRT40A; Act>y^+>Gal4, UAS-GFP (40A tester), y,w, eyFLP1; FRT43D, Tub-Gal80; Act>y^+>Gal4, UAS-GFP (43D tester), y,w, eyFLP1; Act>y^+>Gal4, UAS-GFP; FRT82B, Tub-Gal80 (82B tester), and y,w, eyFLP1; Act>y^+>Gal4, UAS-myrRFP, G454; FRT82B, Tub-Gal80 (82B RFP tester). Additional strains used in this study are described in Supplemental Data. UAS-DTRAF1-IR, UAS-DTRAF2-IR, and a weak allele of UAS-Eiger (UAS-$eiger^W$) were kind gifts of M. Miura. UAS-myrRFP was a kind gift of H. Chang.

Histology

Larval tissues were stained using standard procedures for confocal microscopy. A rabbit anti-β-galactosidase antibody (Cappel, 1:200) and Cy3-conjugated secondary antibody (Jackson Labs) were used. For the analysis of metastatic behavior, the pattern of GFP-expressing mutant cells was carefully observed in the eye discs, brain hemispheres, VNC, and other larval tissues using a Leica MZ FLIII fluorescence stereomicroscope, a BioRad 1240 confocal microscope, and a Zeiss LSM510 META confocal microscope. See also Supplemental Data for additional experimental procedures for histology.

Supplemental Data

Supplemental Data include five figures, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/11/1139/DC1/.

Acknowledgments

We are grateful to M. Miura, E. Kuranaga, and H. Chang for generously provided unpublished reagents. We thank A. Igaki for technical support; S. Kim, L. Pedraza, and J.C. Pastor-Pareja for comments on the manuscript; and the entire Xu lab members for helpful discussions. We also thank T. Adachi-Yamada, T. Aigaki, D. Bilder, E. Kuranaga, B. Lemaitre, M. Miura, M. Nakamura, N. Perrimon, G.M. Rubin, R. Ueda, NIG Stock Center, and Bloomington Stock Center for fly stocks. This work was supported by a grant from NIH/NCI (to T.X.). T.X. is an Investigator of the Howard Hughes Medical Institute. T.I. was supported in part by a fellowship of Yamanouchi Foundation for Research on Metabolic Disorders and is a recipient of the long-term fellowship from the Human Frontier Science Program.

Received: December 19, 2005
Revised: April 12, 2006
Accepted: April 13, 2006
Published: June 5, 2006

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